Interactions between PPxY Motifs in AMOT and WW Domains in NEDD4L Function in Viral Budding

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UROP Proposal

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Abstract

The release of HIV from its host cell requires cooperation with the host ESCRT pathway. This pathway is activated via ubiquitination of HIV Gag. NEDD4L, an important ubiquitin ligase found in humans, is important in the viral release mechanism, but it does not interact directly with HIV like it does with other viruses that use this pathway. Instead, HIV directly binds a human protein called AMOT, which in turn recruits NEDD4L to the plasma membrane. Three PPxY motifs in AMOT appear to interact with four WW domains in NEDD4L, but the nature of their interactions is unknown. Using various biophysical techniques, we propose to determine how the interactions of individual motifs and domains contribute to the affinity of full-length AMOT for full-length NEDD4L. These experiments will further show how HIV and other viruses interact with the ESCRT pathway, and what role ubuiquitination plays in its activation.

Background

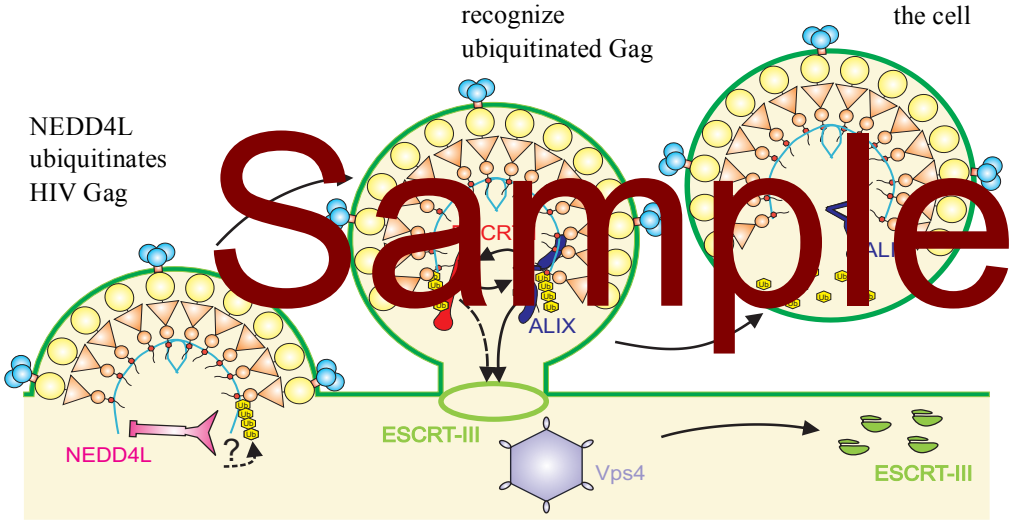
HIV-1 relies on ESCRT (Endosomal Sorting Complexes Required for Transportation) proteins for budding. These proteins recognize and process ubiquitinated proteins, and function in a number of viral pathways (Sette et al., 2013). A variety of ubiquitin ligases found in human cells are important to budding in other retroviruses. In Marburg virus and Ebola virus, for example, NEDD4L, a ubiquitin ligase, performs an important function in the budding process. HIV and other retroviruses require ubiquitination in order to bud from the cell. TSG101, a ubiquitin binding domain in ESCRT-1, has been shown to be instrumental in HIV budding, as has another ubiquitin binding domain, ALIX (Serrano, 2007). The ubiquitination of HIV Gag is therefore important to its interaction with ESCRT proteins.

HIV and NEDD4L interact by both directly binding an intermediate: AMOT (Mercenne et al., 2015). Although it appears that AMOT and NEDD4L are not absolutely necessary for viral

budding, their absence leads to a two-fold reduction in viral release and a five-fold reduction in infectivity. For comparison, the absence of TSG101 leads to a ten-fold decrease in viral release and 20-fold decrease in infectivity, and the absence of ALIX leads to an insignificant reduction of viral release and a 1.5-fold reduction in infectivity. AMOT appears to have a smaller effect than that of TSG101, but a larger effect than that of ALIX.

Cellular depletion of TSG101 leads to an increased arrest of viral budding at the lollipop stage (Figure 1). Cellular depletion of AMOT leads to an increased arrest of viral budding at the half-moon stage, and thus appears to act upstream of TSG101.

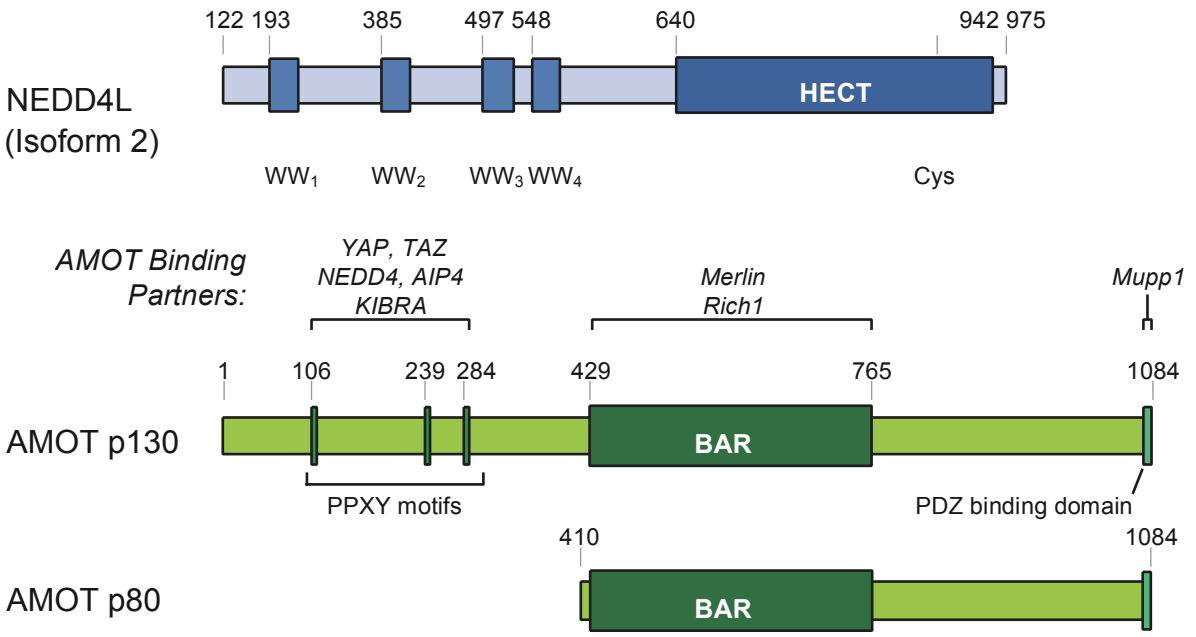
Figure 1: A proposed mechanism for HIV budding



Adapted from: J Votteler Cell Host Microbe. (2013) 14(3):232-41

It is important to note that the block to virus release was rescued when AMOT was re-expressed, but only when a form of AMOT with functional PPxY motifs was used. When AMOT p80 (Figure 2) was re-expressed, viral release was not rescued. Similarly, when a form of AMOT p130 with mutants at each of the PPxY motifs was re-introduced to the cell, viral release was not rescued. Clearly, PPxY interactions with NEDD4L are essential to binding.

Figure 2: Cartoon depiction of NEDD4L and AMOT with key domains shown



Because the ESCRT pathway plays a key role in the budding processes of many viruses, understanding its mechanism is key to understanding the viral life-cycle. NEDD4L interacts directly with some retroviruses other than HIV, and interacts with HIV through AMOT. The goal of this project is to determine how the WW domains in NEDD4L interact with the PPxY motifs in AMOT to produce binding specificity between the two proteins. Understanding how AMOT and NEDD4L interact provides insight into what creates the specificity that allows other viruses to bind NEDD4L directly. Understanding how HIV and other viruses activate the ESCRT pathway could lead to drugs that block that interaction, and lower viral budding rates.

Specific Aim One: To determine specificity in single WW-PPxY interactions.
TIMELINE: IN PROGRESS

NEDD4L and AMOT have been shown to bind each other, but it is unknown what regions of each protein act in binding. This specificity is likely due to interactions between WW

domains in NEDD4L and PPxY motifs in AMOT, and could arise in several ways (Mercenne et al., 2015). Specificity may result from a single interaction between one PPxY motif and one WW domain, or it may result from a set of interactions between multiple motifs and multiple domains. We will determine which WW domains bind which PPxY motifs by purifying single WW domains and single PPxY motifs separate from the rest of their respective proteins. We will use fluorescently-tagged PPxY motifs to perform fluorescent polarization experiments on a serial dilution of WW domains. Preliminary data shows that fluorescent polarization is capable of producing good fits for these interactions (Figure 3 and Table 1). Final fluorescent polarization data will be obtained in triplicate for each possible interaction.

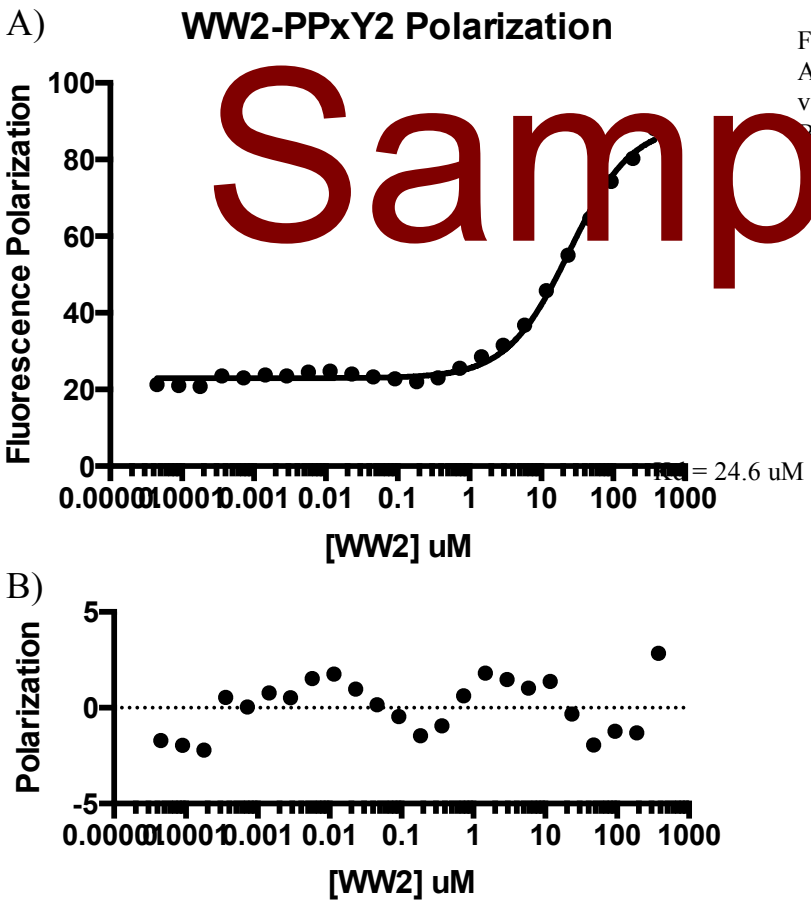


Figure 3:
A) Nonlinear fit of WW2 concentration versus fluorescent polarization
B) Residuals

Table 1: Affinities between individual NEDD4L WW domains and AMOT PPxY motifs.

	WW1	**WW2**	**WW3**	**WW4**
PPxY1	150.2 μM	28.02 μM	6.488 μM	65.67 μM
PPxY2	47.11 μM	24.6 μM	39.21 μM	189.6 μM
PPxY3	>300 μM	>200 μM	119.7 μM	>300 μM

Specific Aim Two: To determine specificity in WW-PPxY interactions in full-length proteins.

TIMELINE: Summer 2015

Preliminary data shows that several interactions appear to have tight binding constants (Table 1). It is unlikely that the entirety of the binding affinity between AMOT and NEDD4L is due to a single interaction between one WW domain and one PPxY motif. We hypothesize that AMOT and NEDD4L interact through some combination of PPxY motifs binding to one or more WW domains. We will test this by performing fluorescence polarization experiments on full-length AMOT and NEDD4L proteins, as well as mutants and double-mutants of those proteins. This experiment will clarify how NEDD4L and AMOT interact biologically. Other work in the lab will show how mutating full-length AMOT and NEDD4L affects budding and infectivity.

Specific Aim Three: To explore the structural biology of important single WW-PPxY interactions and of full-length protein interactions.

TIMELINE: Summer 2015 – Fall 2015

Although the interactions between WW domains and PPxY motifs are well-described, the structural properties behind the interactions of NEDD4L and AMOT are not. We will clarify how these proteins interact by pursuing structures for important single WW-PPxY complexes as well as full-length protein complexes, by using either NMR solution techniques or x-ray crystallography.

Significance

Knowing how AMOT and NEDD4L interact will clarify how HIV and other viruses access the ESCRT pathway, as well as how this pathway functions in normal cell behavior. Ubiquitination of viral core proteins plays an important role in many viral pathways, but HIV appears to have a unique mechanism in that it uses an intermediate protein. By studying how the PPxY motifs in AMOT bind the WW domains in NEDD4L, we will gain insight into interactions between viral PPxY motifs and cellular WW domains. Future drugs could function by blocking the interaction between HIV and AMOT, or the interactions between other viruses like Ebola and NEDD4L.

Dr. [redacted]'s work in the [redacted] lab at the University of Utah has focused on the ESCRT pathway, which HIV uses to bud from the cell. Dr. [redacted] was involved in solving the structure for HIV GAG, and many of the same techniques (i.e. x-ray crystallography) will be applied here to the structure of NEDD4L. Dr. [redacted] also has extensive experience with NMR techniques and other relevant biochemical and biophysical techniques.

I hope to one day get my PhD in some biological field. I believe this research is a good test drive in biochemistry as well as in structural biology. My primary interest lies in infectious diseases, especially HIV. My work in this lab so far as redoubled my interest in HIV, and I believe these experiments will help me figure out from what perspective I want to study HIV.

References

Gaelle Mercenne, Steven L. Alam, Jun Arii, Matthew Lalonde and Wesley I. Sundquist. Angiomotin Functions in HIV-1 Assembly and Budding (2015). eLife. 2015; 4: e03778.

Juan Martin-Serrano (2007). The Role of Ubiquitin in Retroviral Egress. Traffic 2007; 8: 1297–1303.

Paola Sette, Kunio Nagashima, Robert C Piper and Fadila Bouamr (2013). Ubiquitin conjugation to Gag is essential for ESCRT-mediated HIV-1 budding. Retrovirology 2013, 10:79

Shuzo Urata and Jiro Yasuda (2010). Regulation of Marburg virus (MARV) budding by Nedd4.1: a different WW domain of Nedd4.1 is critical for binding to MARV and Ebola virus VP40. Journal of General Virology (2010), 91, 228–234.